Volume 223, number 1, 161–164 FEB 05214 October 1987

Investigations by picosecond polarized fluorescence spectrochronography of structural aspects of energy transfer in living cells of the green bacterium *Chlorobium limicola*

Z.G. Fetisova*, A.M. Freiberg and K.E. Timpmann

*A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Bldg. A, Moscow 119899 and Institute of Physics, Estonian SSR Academy of Sciences, Tartu 202 400, USSR

Received 24 August 1987

The orientation of the long-wavelength (Q_y) transition moments of the antenna bacterioviridin (BVr) was examined in living cells of *Chlorobium limicola*. Previous linear dichroism studies [(1986) FEBS Lett. 199, 234–236] indicated that in each individual chromatophore of *C. limicola* the Q_y transition moment vectors of the whole chlorosome BVr are essentially parallel to each other and are practically ideally oriented along the long axis of the chlorosome. We measured the picosecond polarized fluorescence decay kinetics for antenna bacteriochlorophyll (BChl) emissions upon selective excitation with polarized light of the Q_y transition of BVr. The polarization (p) of the BVr fluorescence is measured to be constant during the BVr excited-state lifetime and to be equal to the limiting value of p achieved in monomeric BChl: $P = +0.42 \pm 0.02$. The results indicate convincingly that the excitation energy transfer within chlorosomes of *C. limicola* cells takes place between chromophores (or their coupled associates) with parallel transition moment vectors.

Antenna; Fluorescence polarization; Energy transfer; Picosecond spectroscopy; Bacterial photosynthesis

1. INTRODUCTION

For various organisms the quantum yield of primary charge separation in the reaction center (RC) is high (~90%) regardless of the size of the photosynthetic unit (PSU), i.e. of the number, N, of antenna molecules per RC. However, such a high quantum yield imposes strong restrictions on the PSU antenna structure [1], which means, in particular, that the antenna structure is to ensure a directed (not random) transfer of excitation energy from an antenna to RC. It is reasonable to assume that in the green sulfur bacterium *Chlorobium limicola* the recently discovered strong orientational ordering of the Q_y transition moments of superantenna bacterioviridin (BVr) [2,3] serves the same task. It was shown with the linear dichroism method that in each *C. limicola* 'chromatophore' the Q_y transition moment vectors of BVr are essentially parallel to each other and practically ideally oriented along the long axis of the chlorosome [2,3]. This orientation was predicted by us on the basis of model computer calculations of the optimal antenna structure [3,4]. If this is the case, i.e. if the excitation transfer within a chlorosome occurs actually via these parallel vectors of BVr transition moments, then it might be expected that the BVr fluorescence anisotropy function, $p(t)$, would be constant during the BVr excited-state lifetime and equal to the limiting value of p achieved in monomeric bacteriochlorophyll (BChl), i.e. +0.42

Correspondence address: Z.G. Fetisova, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Bldg. A, Moscow 119899, USSR

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

[5,6]. To investigate this structural aspect of energy transfer the picosecond polarized fluorescence decay kinetics for antenna BChl emissions in living cells (under physiological conditions) of *C. limicola* were measured upon selective excitation with polarized light of the Q_y transition of BVr.

2. MATERIALS AND METHODS

Cells of *C. limicola* (strain C) were grown as described in [7]. Picosecond fluorescence kinetics were measured by a picosecond spectrochronograph described in [8] in detail. Briefly, the picosecond pulse source was a 'Spectra-Physics' mode-locked CW oxazine 1 dye laser (pulse duration, 3 ps), synchronously pumped at 82 MHz by a krypton-ion laser. The excitation pulses were vertically polarized. Emission, viewed at an angle of 90° to the exciting beam (in a reflection mode), was filtered by two single-grating monochromators (Lomo MDR-1) combined in subtractive dispersion mount to avoid extra pulse broadening (bandwidth of 4 nm) and was registered by a Hamamatsu synchroscan streak camera. The time resolution of the measuring system (FWHM) was 16 ps. For polarization measurements two IR polarizers (K. Zeiss, Jena) were used in the emission beam, with correction for polarization by the detection system. Fluorescence anisotropy values are expressed as $p(t) = (I_\parallel - I_\perp)/(I_\parallel + I_\perp)$, where $I_\parallel$ and $I_\perp$ are the signal intensities $I(t)$ associated with respective fluorescence decay components measured through a polarizer oriented either parallel or perpendicular to the direction of polarization of the exciting light. Positioning of the polarizers was optimized using Rayleigh scattering from a glycogen solution. For the ideal case p should be 1.0; typically, p was 0.98. For data recording and processing, a EC-1010-computer-supported 'B&M Spectronik' OSA 500 optical multichannel analyzer with SIT vidicon is used.

3. RESULTS AND DISCUSSION

All experiments were performed on intact cells 2–5 days old used in their own growth medium under strictly anaerobic conditions. PSUs of green sulfur bacteria contain about 1500 BVr molecules and about 90 BChl *a* molecules per RC P-840, with their main near-infrared absorption peaks at 730–750 and 790–810 nm, respectively. The fluorescence spectra show maxima at 770–785 and ~820 nm, due to BVr and BChl *a* emissions, respectively. Hence, it was necessary to select the BVr and BChl *a* absorption and emission wavelengths that are free of overlapping. As shown in our previous papers [2,3] the 710–770 nm region is the sole BVr absorption one. At the same time, the sole BVr emission region is apparently narrower because of significant overlapping of BVr and BChl *a* fluorescence bands. Therefore, for correct and optimal BVr fluorescence anisotropy measurements the excitation of BVr Q_y transition at $\lambda = 710$ nm was selected and the picosecond BVr fluorescence kinetics were detected at $\lambda = 730$ nm, where contribution from BChl *a* emission is thought to be of minor importance. The BChl *a* fluorescence kinetics were detected at $\lambda = 820$ nm, where contribution from BVr emission is thought to be as small as possible for intact cells at 0.2 W/cm^2 (excitation power used). The kinetics observed were unchanged over an approx. 10^3-fold decrease in the laser pulse intensity. Thus, under our excitation conditions neither singlet-singlet nor singlet-triplet annihilation took place. Figs 1 and 2 show the picosecond polarized fluorescence decay kinetics at room temperature for BVr and BChl *a* emissions, respectively, in living cells of *C. limicola* upon selective excitation of BVr Q_y transition and the calculated fluorescence anisotropy function $p(t)$. According to the data presented in fig.1, the BVr fluorescence anisotropy is constant during the BVr excited-state lifetime and is equal to 0.42 ± 0.02 in the time interval 0–100 ps (or 0.42 ± 0.03 in the time interval 100–270 ps). Our preliminary experiments performed with *C. limicola* 'chromatophores' showed the same results. According to the data presented in fig.2, the BChl *a* fluorescence anisotropy, $p(t)$, fully decayed from its maximum value (+0.42) to approx. zero (−0.02) during the first ~120 ps when the population of BVr excited states decreased about 3-fold and population of BChl *a* excited states achieved its maximum value. We believe that the anisotropy decay lifetime should be regarded as a mean value resulting from several different processes, in particular, because of significant overlapping of BVr

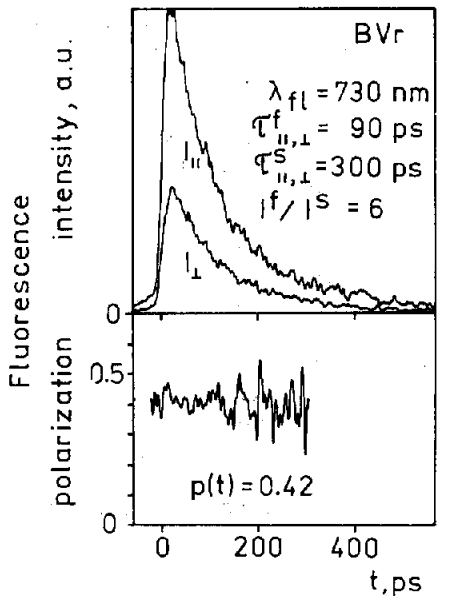

Fig.1. Room-temperature polarized fluorescence kinetics for BVr ($\lambda_{det.}$ = 730 nm) fluorescence decay in living cells of *C. limicola* and the calculated fluorescence anisotropy function, $p(t)$. Excitation with λ = 711 nm was used. Excitation power equals 0.2 W/cm^2. The kinetics were unchanged over an approx. 10^3-fold decrease in the laser pulse intensity. The RCs P-840 are in the closed state at such excitation power [9]. Both kinetics, $I_\parallel$ and $I_\perp$, are strongly biphasic and characterized by a fast phase with a lifetime τ_f = 90 ps and a slow phase with a lifetime τ_s = 300 ps (the amplitude ratio A_f/A_s = 6).

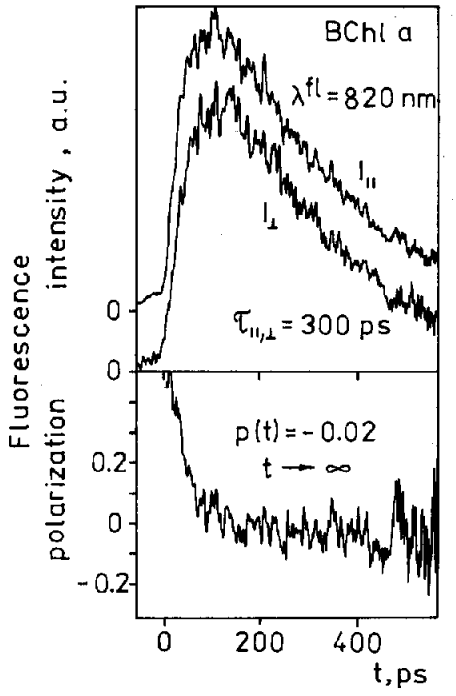

Fig.2. Room-temperature polarized fluorescence kinetics for BChl *a* ($\lambda_{det.}$ = 820 nm) fluorescence decay in living cells of *C. limicola* and the calculated fluorescence anisotropy function, $p(t)$. The experimental conditions are the same as for BVr. Both decay kinetics, $I_\parallel$ and $I_\perp$, are approximated by a single exponent with a time constant τ = 300 ps.

and BChl *a* emission bands at λ = 820 nm during the first 100 ps.

The value of p obtained for BVr emission is equal to the limiting value of p achieved in monomeric BChl. This p value indicates convincingly that the excitation energy transfer within a chlorosome takes place between chromophores (or their coupled associates) with parallel transition moments. It is likely that these transition moments are those of the collective excitation within a cluster of several strongly coupled BVr molecules [10] rather than those of the individual chromophores. Then, the excitation energy transfer within BVr light-harvesting antenna may be described as that between those clusters with parallel transition moments, i.e. each cluster may be considered as a single large 'molecule' which (as a whole) may serve as a donor (an acceptor) molecule in Förster-type excitation transfer (as recognized for the light-harvesting chlorophyll *a/b* protein of higher plants [11]). It is this model which was used in our computations of optimal orientation of BVr dipoles in PSU of *C. limicola* [3]. In any case, the strict orientation of BVr transition moments, discovered by us ([2,3], this work), is one of the optimizing factors ensuring the fast and highly efficient heterogeneous excitation energy transfer from a large BVr antenna to a BChl *a* one that was measured for the first time in our earlier work [12]. Thus, the experimental facts, both the optimal orientational ordering of BVr transition moment vectors and the high degree of BVr fluorescence polarization during its excited-state lifetime, confirm the conclusion of our earlier theoretical work [1]: that the antenna structure should ensure a directed excitation energy transfer from antenna to reaction centers.

REFERENCES

[1] Fetisova, Z.G. and Fok, M.V. (1984) Mol. Biol. (Engl. Transl.) 18, 1354–1359.
[2] Fetisova, Z.G., Kharchenko, S.G. and Abdourakhmanov, I.A. (1986) FEBS Lett. 199, 234–236.
[3] Fetisova, Z.G., Kharchenko, S.G. and Abdourakhmanov, I.A. (1987) in: Progress in Photosynthesis Research (Biggins, J. ed.) vol. 1, pp. 415–418, Martinus Nijhoff Publishers, Dordrecht.
[4] Fetisova, Z.G., Fok, M.V. and Shibaeva, L.V. (1985) Mol. Biol. (Engl. Transl.) 19, 1212–1221.
[5] Bolt, J.D. and Sauer, K. (1981) Biochim. Biophys. Acta 637, 342–347.
[6] Breton, J. and Vermeglio, A. (1982) in: Photosynthesis: Energy Conversion by Plants and Bacteria (Govindjee, ed.) vol. 1, pp. 153–194, Academic Press, New York.
[7] Kondrat'eva, E.N. and Moshentseva, L.V. (1960) Dokl. Akad. Nauk SSSR 135, 460–463.
[8] Freiberg, A.M. and Saari, P. (1983) IEEE J. Quantum Electron. QE-19, 622–630.
[9] Borisov, A.Yu., Fetisova, Z.G. and Godik, V.I. (1977) Biochim. Biophys. Acta 461, 500–509.
[10] Olson, J.M. (1980) Biochim. Biophys. Acta 594, 33–51.
[11] Knox, R.S. and Van Metter, R.L. (1979) in: Chlorophyll Organization and Energy Transfer in Photosynthesis (Wolstenholme, G. and Fitzsimons, D.W. eds) Ciba Foundation Symp. 61, pp. 177–190, Excerpta Medica, Amsterdam.
[12] Fetisova, Z.G. and Borisov, A.Yu. (1980) FEBS Lett. 114, 323–326.